Acta Poloniae Pharmaceutica – Drug Research, Vol. 74 No. 4 pp. 1079–1089, 2017 ISSN 0001-6837
Polish Pharmaceutical Society

DRUG BIOCHEMISTRY

EVALUATION OF THE FAT TISSUE OXIDATIVE STRESS PARAMETERS IN WISTAR RATS IN FRUCTOSE AND HIGH-FAT DIETS WITH THE ADDITION OF *CORNUS MAS L.* AND *ARONIA* JUICE

RENATA FRANCIK[1,2*], MIROSŁAW KROŚNIAK[3], SŁAWOMIR FRANCIK[4], and JOANNA STAWIŃSKA[3]

[1]Department of Bioorganic Chemistry, Jagiellonian University Medical College, 9 Medyczna Street, 30-688 Kraków, Poland
[2]State Higher Vocational School, Institute of Health, Staszica 1 St., 33-300 Nowy Sącz, Poland
[3]Department of Food Chemistry and Nutrition, Jagiellonian University Medical College, 9 Medyczna Street, 30-688 Kraków, Poland
[4]Department of Mechanical Engineering and Agrophysics, Faculty of Production Engineering and Energetics, University of Agriculture in Kraków, 116 B Balicka Street, 30-149 Kraków, Poland

Abstract: Observation of many irregularities in nutrition and disorders of the organ functions, which result from an excessive level of body fat motivates or even imposes the necessity of looking for new ways of prevention and treatment of overweight and obesity. This work presents the effect of food with the addition of fructose (30%), fat (30%) and *Cornus mas* fruit lyophilizate (10%) and *Aronia* juice addition on selected parameters of fat tissue oxidative stress in Wistar rats. The work calculated caloric demand for the tested animals fed with the control, fructose and high-fat diet with and without the addition of *Cornus mas* fruit and *Aronia* juice. In the tested groups the total antioxidative capacity of fat tissue, determined with the FRAP method, the activity of catalase, superoxide dismutase and the level of glutathione were marked. In plasma of the tested animals biochemical parameters were also marked, such as: total cholesterol, concentration of high-density lipoprotein, the level of triglycerides, concentration of urea and uric acid and glucose levels. It was observed in the conducted research, that an addition of *Aronia* juice to the high-fat diet caused a significant increase of UA concentration in the AJ group. *Cornus mas* introduced to the control diet also caused a significant increase of that parameter in the CD group. The conducted experiment shows that the CAT and SOD enzyme activity in the fat tissue homogenate depended on the type of addition introduced to the base feed. The lowest use of feed and the lowest body mass increase was observed in the animal group with high-fat diet and aronia juice (AJ group).

Keywords: catalase, superoxide dismutase, glutathion, *Cornus mas*, *Aronia*, TG, HDL

Proper functioning of a human body requires that a balance is kept between the amount of energy provided to the body in the form of nutritional elements and the amount of energy which is used up. If surplus of energy exceeds the needs for a longer period of time, it begins to be stored in the form of fat tissue. Thus, long-term energy surplus contributes to the development of obesity.

In the last few years, the view on the significance of fat tissue has evolved (1, 2). The fat tissue has been recognized as an active endocrine organ, producing numerous protein substances, such as adiponectin, leptin or A-FABPs (3, 4). The presence in fat tissue of such hormones as leptin or ghrelin is of special importance in the context of obesity pathology, metabolic syndrome or type 2 diabetes.

It is also believed that the A-FABPs proteins play an important role in the pathogenesis of metabolic diseases. An interrelation between increased A-FABP concentration in the pericardial fat tissue and the heart dysfunction in obese patients has been observed. An increase of that protein has been also observed in patients with type 2 diabetes (5, 6).

Fructose contained in diet may present an adverse effect on lipid metabolism in both animals and people (7-10). An increased level of that mono-

* Corresponding author:e-mail: renata.francik@uj.edu.pl; phone: 48 12 6205507; fax: 12 6205693

saccharide may influence in the liver an acceleration of the synthesis of triglycerides (TG) coming from *de novo* lipogenesis (11-13). Carbohydrate and lipid metabolism is connected by the Randle cycle. The carbohydrate – lipid Randle cycle is controlled by insulin and glucagon, hormones regulating the concentration of glucose and fat acids in blood (14, 15). Both an increased amount of carbohydrates as well as branched amino acids in diet stimulate the release of insulin from β cells of Langerhans islets to plasma of the blood. In those conditions intensified processes of glycolysis, glycogenogenesis as well as lipogenesis take place. According to Randle et al. (14), a mitochondrial PDH complex is the main enzyme for the control of the strength of carbohydrate and lipid metabolism.

Obese persons more often suffer from the carbohydrate metabolism disorders. The risk of diabetes increases with the increase of obesity. In this case insulin resistance, and to be more precise, peripheral insulin resistance is of strong significance. A few mechanisms participate in its creation, and first of all intensified lipolysis in fat tissue, forming fat deposits in insulin-sensitive tissues, activity of humoral factors produced by adipocytes, increased dimensions of adipocytes and effects of PPAR receptor stimulation (16).

Relation between the body fat and oxidative stress connected with the amount of reactive oxygen species (ROS) in adipocytes was also demonstrated (7). They are probably ROS that influence the development of inflammation and insulin resistance. To effectively protect tissue cells from oxidative stress, caused by adverse life style and environmental pollution, it would be commendable to introduce in everyday diet such supplements, which will constitute the most effective and safest source of antioxidants. Such supplements may be fruits and vegetables containing compounds decreasing the amount of ROS generated in a living organism (17-19).

Examples of such substances are flavonoids, commonly present in many plants. They significantly decrease the amount of ROS and therefore, they should be an element of daily diet. For most people, consuming each day the amount of fruit or vegetables sufficient to provide the body with 1 g of flavonoids is impossible. Therefore, supplementing the diet with preparations containing flavonoids is necessary (20). Consequently, there is a need to find such diet supplements and such forms, in which they can constitute a valuable addition.

Cornus mas (*Cornelian cherry*) represents a rich source of phenolic antioxidants. It was suggested that Cornelian cherry has very high antiradical activity based on studies of extracts from this plant. Cornelian cherry (*Cornus mas* L.) is one of the most popular plants of the Cornaceae family. Chemical studies showed that cornelian cherry contains a variety of amino acids, organic acids, polysaccharides and saponins, iridoids (21, 22).

Aronia (*Aronia melanocarpa*), which belongs to the rose family (Rosaceae), is one of the plants exhibiting considerable antioxidant potential (23-26). *Aronia* has been used as a dietary supplement in cases of cancer and as anti-inflammatory or antiulcer drug. A positive impact of *Aronia* on animals with experimentally produced diabetes has also been tested. During the study, normalization of body weight and of biochemical parameters of diabetes (including a decrease of glucose level in blood and urine), along with reduction of thirst and amount of urine passed has been observed (27). Compounds present in *Aronia* juice and cornelian cherry fruits are a rich source of anthocyanins and polyphenolic substances, all reducing the amount of free radicals. In this study, was examined the effect of addition of freeze-dried fruit of cornelian cherry or *Aronia* juice to basal diet; diet enriched in carbohydrates/ carbohydrates diet and enriched with fats/ fat diet.

Materials and methods

The experiments were performed in accordance with legal requirements, under a license granted by the Local Commission of Ethics in Kraków number 80/2009 17.09.2009. The experiment was conducted on the 3-month-old male Wistar rats, weighing 250 ± 15 g and caged in the temperature of 23^{o}C, humidity 50–60%, and light dark cycle (12/12 h). Each group consisted of 6 animals.

Animals were fed a diet consisting of a base mix and starch, and with or without the addition of the tested substances. The base mix contained casein (20%), oil (5%), calcium carbonate (2.8%), calcium monohydrogen phosphate (2.9%), lecithin (1%), sodium chloride (0.3%) and minerals and vitamins mix (1%).

The animals in the control group (group CN) were fed the base mix (38%) with the addition of corn starch (62%). The FN group was fed fructose (30%) and corn starch (32%) apart from base mix (38%). The animals in the AN group were fed high-fat diet which consisted of base mix (38%), lard (31%) and corn starch (31%). The animals from the CD, FD and AD groups received on a daily basis an addition of the *Cornus mas* fruit lyophilisate in the form of powder in the amount equal to 10% of the daily base mix for the CN, FN and AN groups.

Fruits of this plant came from an experimental orchard of Agricultural University located in Garlica Murowana. From these fruits, stones were mechanically removed and the obtained part pressed to pulp. The processed material was lyophilized in the LIOGAM factory specializing in freeze drying of fruits and vegetables

The animals in the CJ, FJ and AJ groups, on the other hand, received *Aronia* juice mixed with water to drink (mixed with water in a volume ratio of 3 : 1 (v/v)). The juice was produced by the Eko-Ar Company (it was 100% cold-pressed *Aronia* juice). Moreover, all animals had free access to feed and water.

After 5 weeks, they were euthanized by intraperitoneal injection of sodium thiopental 50 mg/kg. Blood of the animals was collected, from which plasma and fat tissue were extracted. The blood was centrifuged during 15 min (3000 r/min (and frozen until the analysis. Fat tissue was minced in 0.15 M phosphate buffer, pH = 7.4 to 10% final concentration using a basic ultraspeed tissue grinder, the Ultra Turrax T25 homogenizer (12000 r/min bursts). All procedures were performed on ice. Homogenized tissues were centrifuged at 3000 × g for 15 min (0-4^{o}C). The resulting supernatant was drawled and the pellet was discarded. The obtained samples of homogenate were frozen in -80^{o}C until the time of the analysis.

Reagents

All chemicals, solvents, and standards of reagents used in experiments were produced by Sigma-Aldrich. Double-distilled deionized water (Milli-Q, Millipore 18.2 MW/cm 25^{o}C) was used in all experiments.

Biochemical analysis

Biochemical analysis was made with the standard biochemical analyzer Alize B 3.0 with standard kits [total cholesterol (TCHOL), triacylglycerol (TG), uric acid (UA), urea and glucose] from Biomérieux, and it was controlled with Control Serum 1, ODC0003 and Control Serum 2, ODC0004 (OLYMPUS). All the reagents were of analytical grade and were obtained from Sigma Aldrich Chemical Company (Steinheim, Germany).

Determination of total antioxidative activity with the FRAP method

The fat tissue homogenate was marked the FRAP method (Ferric Reducing – Antioxidant Power) described by Benzie and Strain (28). The absorbance in the tested samples was measured with the use of JASCO V-530 spectrophotometer at λ = 593 nm and the FRAP values in 30 min were calculated based on the standard curve.

Determination of glutathion concentration (GSH)

Marking the concentration of glutathion was performer according to Ellman method (29). The method uses the reduction of DTNB by thiole complexes (e.g., glutathione).

Determination of catalase activity (CAT)

The activity of catalase was determined according to the Aebi method (30), with the use of the JASCO V-530 spectrophotometer. The absorbance was measured for 3 min, at λ = 240 nm at 25^{o}C. From the standard curve the value of CAT activity was calculated in commutation to protein [U/mg of protein]. One unit of CAT activity was defined as the amount of enzyme decomposing 1 μM of H_2O_2 per min.

Determination of superoxide dismutase (SOD) activity

The activity of superoxide dismutase was marked according to Flohe and Otting method (31). In this method the source of the superoxide anion is the reaction of xanthine oxidase, and the indicator reaction is the reduction of cytochrome C, the absorbance of which was recorded at 550 nm wavelength. Measurements were done in cuvettes to which the following solution was added: 950 μL of buffer for marking SOD containing: 50 μM of xanthine, 20 μM of cytochrome C, 50 mM of potassium-phosphate buffer pH 7.8 and 0.1 mM EDTANa3, and 30 μL of fat tissue homogenate. The measurement started by adding 20 μL of the xanthine oxidase solution and conducted in the temperature of 30^{o}C. The SOD activity was marked based on the linear reading of changes of absorbance between the 15th s and the second min and 15th s at 550 nm wavelength, and it was expressed in the specific activity units (U), per 1 mg of protein (U/mg protein).

Statistics

Statistical analysis was conducted with STATISTICA PL v.7.1 (StatSoft, Tulsa, USA). The significance of statistical differences for biochemical parameters in different animal groups was checked on the basis of the non-parametric test ANOVA rank by Kruskal-Wallis. The value of $p < 0.05$ was adopted as a critical significance level. Calculations for the Spearman correlation were also made with the use of the same software.

RESULTS AND DISCUSSION

Dietary fructose consumption is one of the environmental factors contributing to the development of obesity (32-34). Treatment of obesity is currently based mainly on introducing physical activity and modifying the diet, and thus, non-pharmacological treatment. It is, therefore, important to determine and analyze the effect of dietary modifications on the fat tissue function, its biochemical parameters and on body mass reduction.

Obesity is now among frequently discussed topics, especially in the developed countries, where it poses the biggest problem. Namely, it entails a range of complications, among others an increased risk of metabolic and circulatory system diseases. The dynamics of progression of obesity and carbohydrate metabolism use disorder has caused a growing interest in the fat tissue function and the activity of antioxidative parameters present in this tissue (35, 36).

These days, in the era of obesity, in fact only a few publications are our source of knowledge on the activity of enzymes and hormones in adipocytes, on antioxidative potential or changes in fat tissues as a result of various types of diets. This situation is caused primarily by difficulties in isolating and marking enzymes, and by low concentration of

Table 1. Use of feed and energetic balance in the tested animal groups.

Animal group	Average use of feed [g/5 weeks]	Caloric value of feed [kcal/g feed]	Average body mass growth [g/animal]	Energetic balance [kcal/g mass]
CN	657.7	3.827	119.3 ± 18.5	21.09
CD	642.3	3.727	118.2 ± 13.1	20.25
CJ	593.80	3.727	113.8 ± 20.2	19.96
FN	624.69	3.827	128.5 ± 9.4	18.60
FD	652.32	3.727	108.5 ± 19.9	22.41
FJ	667.75	3.727	116.2 ± 36.7	21.99
AN	471.55	5.387	129.0 ± 21.2	19.69
AD	489.12	5.387	140.4 ± 28.8	18.42
AJ	416.28	5.387	93.2 ± 7.2	24.06

(CN) - control; (FN) - fructose; (AN) - high-fat; (CD) - cornelian cherry with control; (CJ) - chokeberry juice with control (FD) - cornelian cherry with fructose; (FJ) - chokeberry juice with fructose; (AD) - cornelian cherry with high-fat; (AJ) - chokeberry juice with high-fat.

Table 2. Activity of oxidative stress markers (FRAP, CAT, SOD, GSH) marked in fat tissue homogenates in Wistar rats.

X1-diet	X2-supplement	FRAP [mM/ mg protein]	CAT [U/ mg protein]	GSH [nM/mg protein]	SOD [U/ mg protein]
C	**N**	0.53 ± 0.18^{A}	0.354 ± 0.127^{AB}	0.058 ± 0.026^{A}	4.97 ± 1.83^{ABC}
C	**D**	0.72 ± 0.1^{A}	0.252 ± 0.128^{A}	0.288 ± 0.263^{ABC}	2.26 ± 0.59^{AB}
C	**J**	1.07 ± 0.52^{AB}	0.181 ± 0.047^{A}	0.172 ± 0.073^{A}	2.11 ± 0.62^{A}
F	**N**	0.68 ± 0.32^{A}	0.557 ± 0.241^{B}	0.063 ± 0.028^{A}	2.7 ± 1.39^{AB}
F	**D**	1.17 ± 0.59^{AB}	0.34 ± 0.198^{AB}	0.55 ± 0.374^{BC}	5.74 ± 2.68^{ABC}
F	**J**	1.94 ± 0.89^{B}	0.176 ± 0.085^{A}	0.569 ± 0.208^{C}	2.55 ± 1.24^{AB}
A	**N**	0.97 ± 0.29^{A}	0.243 ± 0.098^{A}	0.279 ± 0.093^{ABC}	8.68 ± 2.77^{C}
A	**D**	1.01 ± 0.46^{A}	0.241 ± 0.037^{A}	0.211 ± 0.053^{AB}	7.38 ± 4.41^{C}
A	**J**	0.47 ± 0.15^{A}	0.256 ± 0.151^{A}	0.116 ± 0.038^{A}	7.01 ± 3.03^{BC}

(CN) – control; (FN) – fructose; (AN) – high-fat; (CD) – cornelian cherry with control; (CJ) – chokeberry juice with control (FD) – cornelian cherry with fructose; (FJ) – chokeberry juice with fructose; (AD) – cornelian cherry with high-fat; (AJ) – chokeberry juice with high-fat. Data are presented as the means from independent measurements ± standard deviation (SD). Different letters in the same columns indicate significant differences according to Tukey's test ($p < 0.05$).

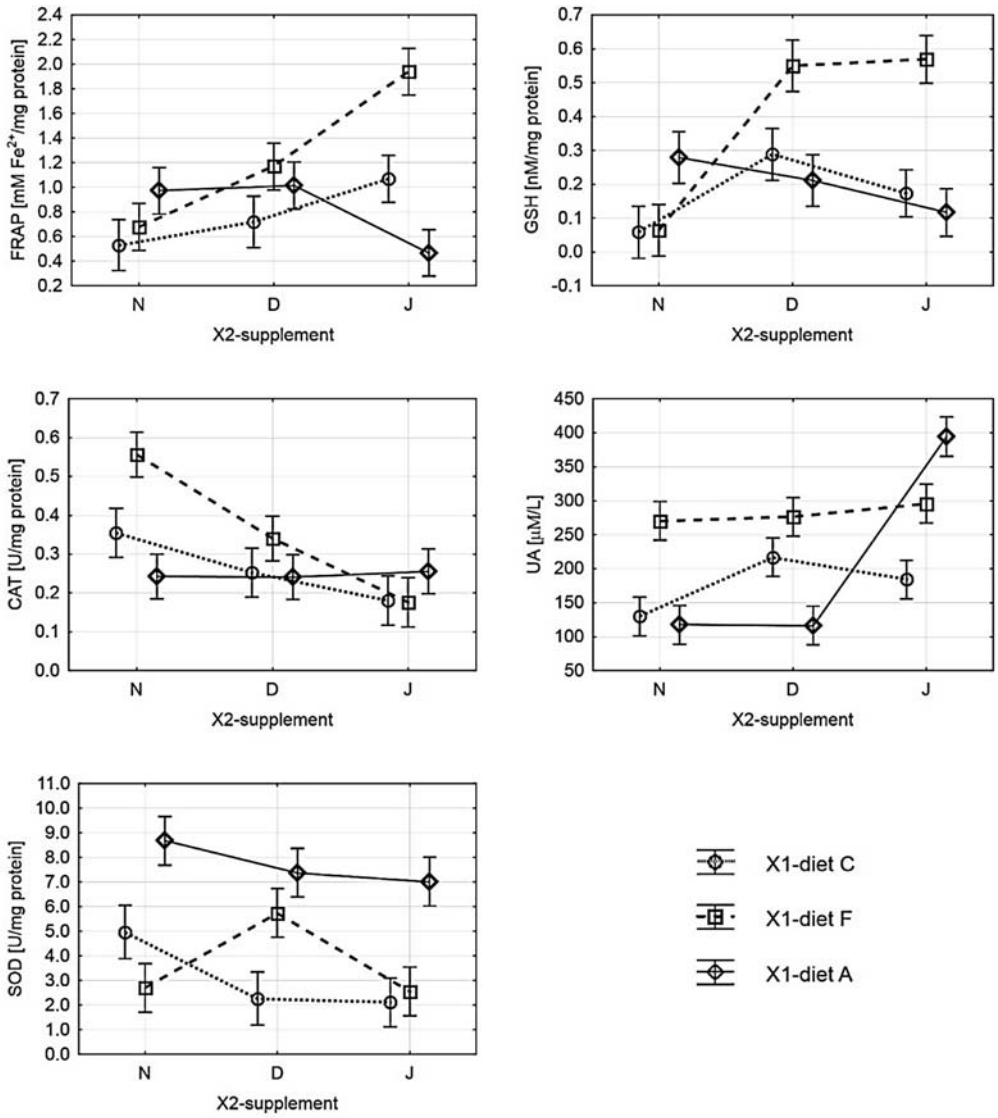

Figure 1. Antioxidant parameters in fat tissue of rats in different types of diets (control, fructose, high-fat) without or with fruits of cornelian cherry or chokeberry juice (N – without supplement; D – cornelian cherry; J – chokeberry juice)

those complexes in fat tissue. Many authors have described effects of diet on body mass. Kuroshima (37) found out that there was a lack of statistically significant changes in the body mass growth of rats fed with a diet with the addition of 32% sucrose solution in comparison to the control diet.

Based on the results obtained for our research model, combination of high-fat diet with *Cornus mas* fruit proved adverse, as the significant body mass increase occurred in the tested group. Supplementing that diet with *Aronia* juice caused the lowest body mass increase. Combination of *Cornus mas* or *Aronia* with the fructose diet also resulted in a significant decrease of the body mass growth in the tested animals.

Average use of feed per animal during the 5-weeks experiment as well as calorific value of the applied diet is presented in Table 1. Average body mass increase and the amount of calories needed for an increase of one gram of body mass depending on the applied diet is also shown. The lowest use of feed and the lowest body mass increase was observed in the animal group with high-fat diet and *Aronia* juice (AJ group). At the same time the animals in that group received the feed with the highest calorific value. In the FD group we observed

increased use of feed in comparison to the FN group but a decreased body mass growth, which may suggest the influence of *Cornus mas* on food absorption. In the rats fed with the fructose diet (FN) a higher body mass growth was observed in comparison to the control group.

Obesity and body mass growth cause increased generation of free radicals in fat tissue (38). This in turn causes an increase of the total antioxidative activity, marked as FRAP. The presence of polyphenols and anthocyanins is directly related to the antioxidative properties. It is possible that polyphenols are responsible for the FRAP value increase in the diets with the addition of *Cornus mas* or *Aronia* (24, 25, 39).

It was observed in the conducted research model that the high-fat diet increased the FRAP (Table 2, Fig. 1) value in a statistically significant way in comparison the control diet. *Cornus mas* – introduced to the feed only the fructose diet (FD group) caused a statistically significant increase of the FRAP value in comparison to the fructose diet. Additionally, it was observed that *Aronia* juice combined with the high-fat diet statistically significantly decreased the FRAP value in comparison with the pure high-fat diet.

Based on the conducted experiment one can draw a conclusion that both *Cornus mas* and *Aronia* served with an increased dose of fructose increase the antioxidant capacity of fat tissue. However, the combination of *Aronia* juice with the high-fat diet had an adverse effect on the value of that parameter, causing its statistically significant decrease.

The capacity of the complexes present in *Cornus mas* to level RFT was also demonstrated by the Ersoy et al. (39) research results. They compared 12 species of *Cornus mas* with regard to the antioxidative capacities of the methanol extract from fruits.

The first line of defense is preventing damages of biological particles by preventing the generation of the most reactive among RFT (e.g., radical hydroxyl). A special role in removing dangerous free radicals is played by enzymatic mechanisms (the activity of SOD and CAT), as well as by some non-enzymatic ones (GSH or UA).

Glutathione (GSH) plays the role of intracellular redox buffer of big capacity and decreases the pool of reactive electrophile compouds. It constitutes the main element of defense against antioxidative stress. The concentration of intracellular glutathione is particular for a given type of cells and it ranges from 5 to 10 mM (40). It is not constant and may undergo a significant decrease, among others in the organ cells of aging organisms as a result of oxidation to GSSG, increased degradation and decreased productions (41, 42).

The level of reduced GSH (Tab. 2, Fig. 1) in the high-fatty group (AN) was statistically significantly higher than in the control group (CN) or fructose group (FN). Adding *Cornus mas* and *Aronia* to the food rich in fructose caused a statistically significant increase of the GSH value in comparison to the F group. *Cornus mas* added to the control diet in comparison to the control diet itself influenced the statistically significant increase of glutathione.

The decrease of GSH concentration in the liver cells may be the result of oxidative stress induction. The decreased GSH value may be the cause of changes in the functioning of cell membranes due to the peroxidation of membrane lipids. In the conducted experiment an addition of *Cornus* or *Aronia* to the fructose diet was observed to cause a significant increase of the GSH level in the fat tissue.

Galinier showed that fat tissue of obese rats contained a higher concentration of hydrophilic antioxidants, such as glutathione or vitamin C (43, 44). The increase of glutathione concentration as a result of the diet is low and short-term, but still it may have enormous role in the detoxication of free radicals and in the defense of cells against oxidative stress. The addition of *Cornus* fruits to the control diet significantly increased the amount of glutathione in the fat tissue. Combining *Cornus* or *Aronia* with the fructose diet also caused a statistically significant increase of glutathione concentration in the tested tissue.

The enzyme catalase (CAT) plays a role in the protection of fat tissues from the toxic effects of H_2O_2 and partially reduced oxygen species. Catalase, iron-containing enzyme (oxidoreductase) which catalases the breakdown of H_2O_2 is a potentially destructive agent in cells. In the animal group with the feed enriched with fructose, the highest activity of CAT (Table 2, Fig. 1) was marked, it was statistically significantly higher than in the CN group and AN group. It was observed that the addition of *Cornus mas* or *Aronia* juice to the FN diet caused a statistically significant decrease of the CAT activity in comparison to the F group.

Galinier (43) based on the conducted research determined that obesity has no influence on the Mn-SOD and Cu/Zn-SOD activity but in the obese rats they recorded an increase of the catalase activity. In fat tissue marked the activity of basic antioxidative enzymes in fat, namely of CAT, glutathione peroxidase (GPx) and SOD activity (44). In obese rats there were significant linkages between obesity and

oxidative stress, and thus, also body defense mechanism, which are demonstrated by increased production and activity of antioxidants (43). In the conducted experiment, the SOD activity (Table 2, Fig. 1) was observed to be the highest and statistically significant in the animal group with high-fat diet in relation to the CN group and FN group, in which the lowest SOD activity value was observed. Adding *Cornus mas* to the fructose feed caused a statistically significant increase of the SOD activity in comparison to the FN group.

Adding *Cornus mas* or *Aronia* to the control feed in the animals in the CD group and the CJ group decreased the SOD activity in a statistically significant way in comparison to the CN group. In the fructose diet *Cornus mas* increased the SOD activity while *Aronia* (FJ group) did not influence the activity of the tested enzyme in comparison to the FN group.

The conducted experiment shows that the CAT and SOD enzyme activity in the fat tissue homogenate depended on the type of addition introduced to the base feed. Fructose caused increased CAT activity with a simultaneous decrease of SOD activity in comparison to the control group. Fat, on the other hand, worked in an opposite way; namely,

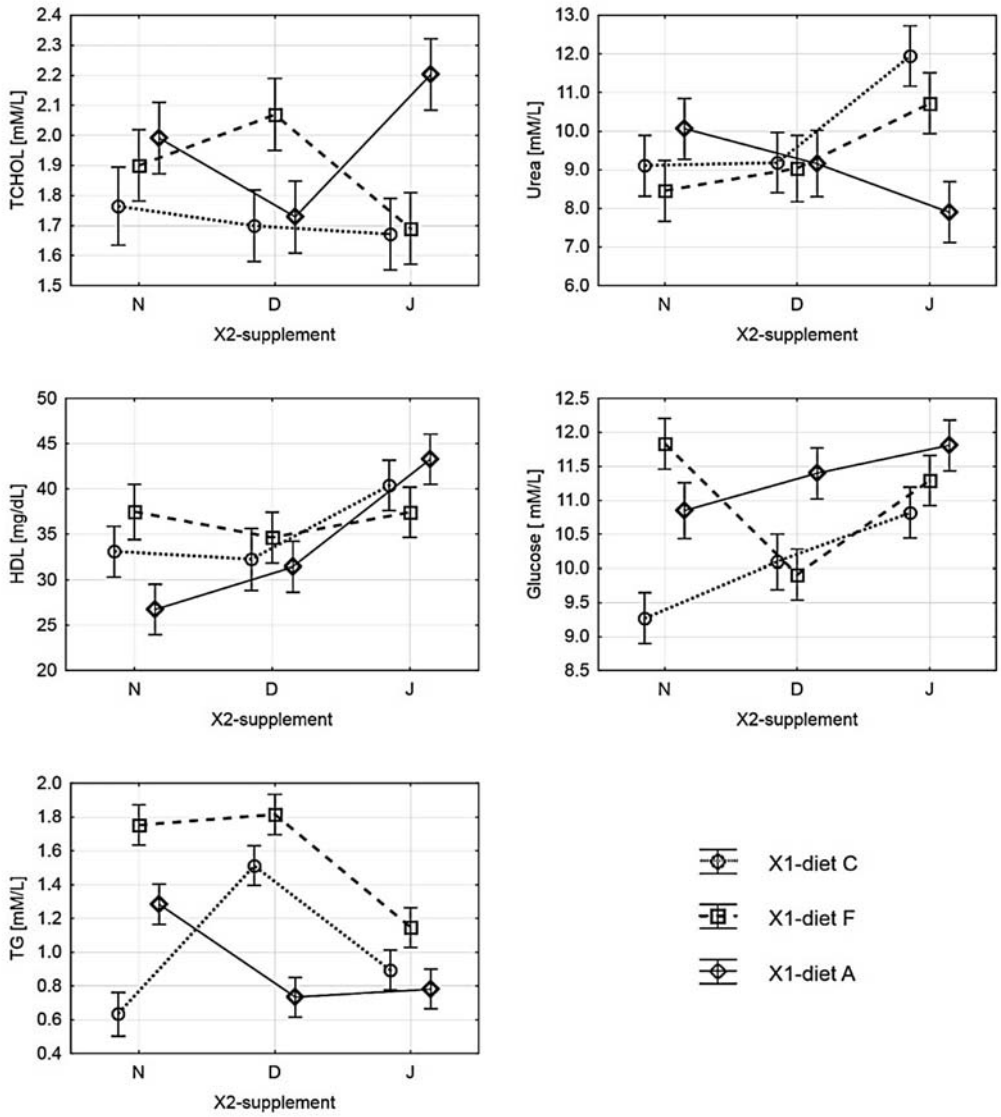

Figure 2. Concentration of total cholesterol (TCHOL); high-density lipoprotein (HDL), triglycerides (TG); urea and glucose in plasma of rats in different types of diets (control, fructose, high-fat) without or with fruits of cornelian cherry or chokeberry juice (N – without supplement; D – cornelian cherry; J – chokeberry juice)

it decreased CAT activity while significantly increasing SOD activity. Adding *Cornus mas* or *Aronia* to the fructose diet caused a significant decrease of CAT activity. In the high-fat diet no influence of *Cornus mas* or *Aronia* on CAT activity was observed. As for the control diet, SOD activity decreased as a result of adding *Cornus* or *Aronia*.

Fructose is metabolized only in the liver where it enters the Embden–Meyerhof–Parnas (EMP) pathway after phosphorylation with the participation of fructokinase enzyme. Liver catabolism of that monosacharide to fat acids includes the conversion of pyruvate synthetized in glycolyse pathway to acetylo-CoA with the participation of pyruvate dehydrogenase complex (PDH). A few mechanisms take part in its generation, and first of all intensified lipolyse in fat tissue, cumulating/fat deposits in the tissue dependent to insulin, the activity of humoral factors produced by adipocytes, increased size of adipocytes and the effects of PPAR receptor stimulation (16). Additionally, circulating levels of A-FABP are closely correlated with several key features of the metabolic syndrome, including adverse lipid profiles (increased serum triglyceride and LDL-cholesterol, and decreased HDL-cholesterol), insulin resistance, hyperglycemia and hypertension, independent of sex, age, and obesity. There is a significant increasing trend in A-FABP levels with the increasing number of components of the metabolic syndrome (45). As for biochemical analysis conducted in the obtained plasma, the level of lipids such as TCHOL, HDL and TG was marked (Table 3, Fig. 2). It was observed that the duration of the experiment did not cause significant changes in the cholesterol concentration in the FN and AN groups in comparison to the CN group. Besides, neither the tested additions of *Cornus mas* fruit nor *Aronia* juice had a statistically significant effect on the tested parameter value. The animal group with the high-fat diet with *Aronia* juice had a statistically significantly increased level of TCHOL in comparison to the CJ group or FJ group.

In the group of animals with high-fat diet with *Aronia* addition, there was a statistically significant increase of the HDL level in comparison to the AN group. Also *Aronia* introduced to the control feed had a similar effect on the HDL level in comparison to the CN group.

Busserolles (13) demonstrated that diet rich in fructose causes hypertriglycerydemia. The fructose content in the food used in the above mentioned research was similar (34%) to the model which was used in our experiment (30% of fructose). However, probably due to the duration of the experiment, no influence of the applied diets was observed on the cholesterol concentration in the blood plasma.

At the same time, changes in TG and HDL concentration were demonstrated. The level of TG marked in the FN group was statistically significantly higher than in the CN group and the AN group. The addition of *Cornus mas* decreased the level of TG in a statistically significant way only in the high-fat diet in comparison to the AN group. The TG value in the AD group was the lowest statistically significant only in comparison to the CD and FD groups. In the CD group a statistically significant TG increase was observed in comparison to the CN group. Adding *Aronia* to the fructose diet and high-

Table 3. Biochemical parameters in plasma of Wistar rats.

X1- diet	X2- supplement	TCHOL [mM/L]	HDL [mg/dL]	TG [mM/L]
C	**N**	1.76 ± 0.04^{A}	33.11 ± 6.63^{AB}	0.634 ± 0.196^{A}
C	**D**	1.7 ± 0.17^{A}	32.23 ± 0.87^{AB}	1.513 ± 0.27^{CD}
C	**J**	1.67 ± 0.17^{A}	40.38 ± 6.28^{A}	0.895 ± 0.206^{AB}
F	**N**	1.9 ± 0.31^{A}	37.46 ± 5.93^{AB}	1.752 ± 0.235^{C}
F	**D**	2.07 ± 0.43^{A}	34.62 ± 7.61^{AB}	1.816 ± 0.342^{C}
F	**J**	1.69 ± 0.26^{A}	37.43 ± 5.11^{AB}	1.147 ± 0.175^{ABD}
A	**N**	1.99 ± 0.48^{A}	26.73 ± 8.46^{B}	1.283 ± 0.544^{BCD}
A	**D**	1.73 ± 0.08^{A}	31.42 ± 1.62^{AB}	0.733 ± 0.272^{A}
A	**J**	2.2 ± 0.33^{A}	43.27 ± 11.05^{A}	0.781 ± 0.121^{AB}

(CN) – control; (FN) – fructose; (AN) – high-fat; (CD) – cornelian cherry with control; (CJ) – chokeberry juice with control (FD) – cornelian cherry with fructose; (FJ) – chokeberry juice with fructose; (AD) – cornelian cherry with high-fat; (AJ) – chokeberry juice with high-fat. Data are presented as the means from independent measurements ± standard deviation (SD). Different letters in the same columns indicate significant differences according to Tukey's test ($p < 0.05$).

Table 4. Biochemical parameters in plasma of Wistar rats.

X1- diet	X2- supplement	UA [μM/L]	Urea [mM/L]	Glucose [mM/L]
C	**N**	130 ± 64^{A}	9.11 ± 2.22^{AB}	9.27 ± 0.86^{C}
C	**N**	130 ± 64^{A}	9.11 ± 2.22^{AB}	9.27 ± 0.86^{C}
C	**J**	184 ± 74^{AB}	11.95 ± 2.58^{B}	10.82 ± 0.79^{ABC}
F	**N**	270 ± 43^{BC}	8.46 ± 1.72^{AB}	11.83 ± 1.17^{A}
F	**D**	276 ± 73^{BC}	9.03 ± 1.26^{AB}	9.91 ± 1.24^{BC}
F	**J**	296 ± 75^{BC}	10.72 ± 1.52^{AB}	11.29 ± 0.85^{AB}
A	**N**	117 ± 30^{A}	10.06 ± 1.82^{AB}	10.85 ± 0.26^{ABC}
A	**D**	116 ± 39^{A}	9.16 ± 0.28^{AB}	11.4 ± 0.7^{AB}
A	**J**	394 ± 124^{C}	7.9 ± 2.11^{A}	11.81 ± 0.96^{A}

(CN) – control; (FN) – fructose; (AN) – high-fat; (CD) – cornelian cherry with control; (CJ) – chokeberry juice with control (FD) – cornelian cherry with fructose; (FJ) – chokeberry juice with fructose; (AD) – cornelian cherry with high-fat; (AJ) – chokeberry juice with high-fat. Data are presented as the means from independent measurements ± standard deviation (SD). Different letters in the same columns indicate significant differences according to Tukey's test ($p < 0.05$).

fat diet resulted in a decrease of the TG level in comparison to the groups FN and AN.

In the high-fat diet, *Aronia* juice (AJ group) significantly influenced the decrease of TG concentration and at the same time caused an increase of HDL concentration. Introducing *Aronia* juice in the fructose diet (FJ group) also caused a statistically significant decrease of TG concentration. Combining *Aronia* juice with the control diet (CJ group) caused a significant increase of HDL in the blood plasma. Adding *Cornus* to the control diet (CD group) caused a significant increase of the TG level. In the high-fat diet an opposite effect was observed: *Cornus mas* caused a significant TG decrease.

Obesity, hypertriglyceridemia, hypertension and insulin resistance especially strongly increase the production and retention of uric acid (UA) (46). Hayden et al. (46) demonstrated that hyperglycemia and decreased concentration of HDL fraction cholesterol also show a positive correlation with the UA concentration. Additionally, the works of Hikita (47) draw attention to the correlation between UA concentration and fat acids distribution. It was also demonstrated that UA concentration is influenced by leptin — a hormone produced by adipocytes. It is believed that it may be one of the factors conditioning the occurrence of hyperuricemia connected with adipose (48).

In the blood serum, the levels of UA, urea and glucose were also marked. Uric acid (Table 4, Fig. 1) generated in the blood serum of the animals with the fructose diet had a statistically significantly higher level than in the CN group and the AN group. In the CD group a statistically significant increase of UA was observed in comparison to the CN group. Adding *Cornus mas* to the other diets (FN and AN) did not cause statistically significant changes in the UA level. Introducing *Aronia* juice in the feeding of animals with the high-fat diet caused a statistically significant increase of the UA concentration in comparison to the AN group.

It was observed in the conducted research that an addition of aronia juice to the high-fat diet (AJ group) caused a significant increase of UA concentration. *Cornus* introduced to the control diet also caused a significant increase of that parameter in the CD group. Increased uric acid concentration is commonly observed in the persons with the, so called, life style diseases: obesity, hypertension, diabetes, blood lipids disorders, arteriosclerosis

The level of urea marked in the tested animal groups was influenced by *Aronia* juice (Table 4, Fig. 2). In the blood serum the highest level was marked in the CJ group and it was statistically significant in comparison to the CN group. In the AJ group a statistically significant decrease of the urea level in comparison to the AN group was observed. *Aronia* juice introduced to the control (CJ) and fructose diets caused a statistically significant increase of the measured parameter in comparison to the CN and FN diets.

The level of glucose (Table 4, Fig. 2) in the blood serum of the FN animal group was statistically significantly higher than in the CN groups. In the FD group there was a statistically significant decrease of the glucose value in comparison to the FN group. In the animals with the control feed with

the addition of *Aronia* a statistically significant increase of the glucose concentration in comparison to the CN group was observed.

Dyslipidemia (including decreased concentration of HDL fraction cholesterol and increased plasma triglycerides levels) and uric acid metabolism disorders are the disorders characteristic of the insulin resistance syndrome (49). Introducing such supplements as *Cornus* fruit or aronia juice to everyday diet may have influence on the proper level of antioxidative balance in tissues.

CONCLUSION

The disruption of antioxidant balance of the body system is an important factor in development of many diseases, including type 2 diabetes or metabolic syndrome. *Cornus mas* and aronia juice contains many substances with antioxidant properties. Fructose and fat diet encourages faster weight gain relative to the control diet.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

We would like to express our gratitude to Krzysztof Ryt for the lyophilization of samples. This work was supported in part by Department of Food Chemistry and Nutrition K/ZDF/004144 and in part by Ministry of Science and Higher Education, Poland (statutory activities DS-3600/WIPiE/2014, Faculty of Production and Power Engineering, University of Agriculture).

REFERENCES

1. Uchida A., Slipchenko M.N., Cheng J.X., Buhman K.K.: Biochim. Biophys. Acta 1811, 170 (2011).
2. Oller de Nascimento C.M., Ribeiro E.B., Oyama L.M.: An. Acad. Bras. Cienc. 81, 453 (2009).
3. Coelho M., Oliveira T., Fernandes R.: Arch. Med. Sci. 2, 191 (2013).
4. Kershaw E.E., Flier J.S.: J. Clin. Endocrinol. Metab. 89, 2548 (2004).
5. Kralisch S., Ebert T., Lossner U., Jessnitzer B., Stumvoll M. et al.: Int. J. Obes. 38, 1251 (2014).
6. Tso A.W., Xu A., Sham P.C., Wat N.M., Wang Y. et al.: Diabetes Care 30, 2667 (2007).
7. Pawłowska J., Witkowski J.M., Bryl E.: Forum Med. Rodz. 3, 222 (2009).
8. Sundaram R., Shanthi P., Sachdanandam P.: Mol. Cell. Biochem. 380, 43 (2013).
9. Stanković M.N., Mladenović D.R., Duričić I., Šobajić S.S., Timić J. et al.: Arch. Med. Res. 45, 116 (2014).
10. Zhou A.L., Hintze K.J., Jimenez-Flores R., Ward R.E.: Lipids 47, 1119 (2012).
11. Paśko P., Bartoń, H., Zagrodzki P., Izewska A., Krośniak M. et al.: Plant Foods Hum. Nutr. 65, 146 (2010).
12. Tappy L., Le K.A., Tran C.H. Paquot N.: Nutrition 26, 1044 (2010).
13. Busserolles J., Gueux E., Rock E., Demigne C., Mazur A. et al.: J. Nutr. 133, 1903 (2003).
14. Randle P.J., Garland P.B., Hales C.N., Newshlome F.A.: Lancet 281, 785 (1963).
15. Zhou Y.P., Priestman D.A., Randle P.J., Grill V.E.: Am. J. Physiol. 270, E988 (1996).
16. Krassowski J.: Endokrynol. Pol. 4, 483 (2004).
17. Matkowski A., Kuś P., Góralska E., Woźniak D.: Mini Rev. Med. Chem. 13, 439 (2013).
18. Xu Z.R., Li J.Y., Dong X.W., Tan Z.J., Wu W.Z. et al.: Nutrients 24, 7085 (2015).
19. Tundis R., Loizzo M.R., Menichini F., Bonesi M., Conforti F. et al.: Plant Foods Hum. Nutr. 66, 261 (2011).
20. Miano L.: Arch. Ital. Urol. Androl. 75, 166 (2003).
21. Krośniak M., Gąstoł M., Szałkowski M., Zagrodzki P., Derwisz M.: J. Toxicol. Env. Health A. 73, 1155 (2010).
22. Tural S., Koca I.: Sci. Hortic-Amsterdam. 116, 362 (2008).
23. Slimestad R., Torskangerpoll K., Nateland H.S., Johannessen T., Giske N.H.: J. Food Compos. Anal. 18, 61 (2005).
24. Valcheva-Kuzmanova S., Marazova K., Krasnaliev I., Galunska B., Borisova P. et al.: Exp. Toxicol. Pathol. 56(6), 385 (2005).
25. Bijak M., Bobrowski M., Borowiecka M., Podsędek A., Golański J. et al.: Fitoterapia 82, 811 (2011).
26. Kokotkiewicz A., Jaremicz Z., Luczkiewicz M.: J. Med. Food. 13, 255 (2010).
27. Saluk-Juszczak J.: Postep. Hig. Med. Dosw. 64, 451 (2010).
28. Benzie I.F., Strain J.J.: Anal. Biochem. 239, 70 (1996).
29. Ellman G.L.: Arch. Biochem. Biophys.74, 443 (1958).

30. Aebi H.: Methods. Enzymol. 105, 121 (1984).
31. Flohe L., Otting F.: Methods. Enzymol. 105, 93 (1984).
32. Schaefer E.J., Gleason J.A., Dansinger M.L.: J. Nutr. 139,1257S (2009).
33. Tappy L., Lę K.A.: Physiol Rev. 90, 23 (2010).
34. Gaby A.R.: Altern. Med. Rev. 10, 294 (2005).
35. Kralisch S., Klein J., Lossner U., Bluher M., Paschke R. et al.: J. Endocrinol. 185, R1 (2005).
36. Kershaw E.E., Flier J.S.: J. Clin. Endocrinol. Metab. 9, 2548 (2004).
37. Kuroshima A., Ohno T., Moriya M., Ohinata H., Yahata T. et al.: J. Therm. Biol. 20, 477 (1995).
38. Olszanecka-Glinianowicz M., Zahorska-Markiewicz B.: Postepy Hyg. Med. Dosw. 62, 249 (2008).
39. Ersoy N., Bagci Y., Gok V.: Sci. Res. Essays. 6, 98 (2011).
40. Meister A., Anderson M.E.: Ann. Rev. Biochem. 52, 711 (1983).
41. Öztürk O., Gümüşlü S.: Exp. Gerontol. 39, 211 (2004).
42. Yu B.P., Chung H.Y.: Diabetes Res. Clin. Pract. 54, S73 (2001).
43. Galinier A., Carrière A., Fernandez Y., Carpéné C., André M. et al.: J. Biol. Chem. 281, 12682 (2006).
44. Galinier A., Carriere A., Fernandez Y., Caspar-Bauguil S., Periquet B. et al.: FEBS Lett. 580, 6391 (2006).
45. Xu A., Wang Y., Xu J.Y., Stejskal D., Tam S. et al.: Clin. Chem. 52, 405 (2006).
46. Hayden M.R., Tyagi S.C.: Nutr. Metab. 1, 10 (2004).
47. Hikita M., Ohno I., Mori Y., Ichida K., Yokose T. et al.: Intern. Med. 46, 1353 (2007).
48. Chen L.Y., Zhu W.H., Chen Z.W., Dai H.L., Ren J.J. et al.: J. Zhejiang Univ. Sci. B. 8, 593 (2007).
49. Wilcox G.: Clin. Biochem. Rev. 26, 19 (2005).

Received: 1. 07. 2016